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Theoretical calculations of base-base interactions in nucleic acids: I Stacking interactions in free bases

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### ABSTRACT

Stacking interactions in free bases were computed on the basis of molecular association. The results of the calculations were compared with the stacking patterns observed in a few single crystals of nucleic acid components as examples. The following are the conclusions: (i) there can be two types of stacking pattern classified as normal and inverted types for any two interacting bases and both can be energetically favourable (ii) in both the types the stacking interaction is a combined effect of the overlap of the interacting bases and relative positions and orientations of the atomic centres of the two bases (iii) crystal symmetry and H-bonding interaction may influence stacking patterns.

### INTRODUCTION

It is well known that base-base interactions play a dominant role in stabilizing the secondary structure of nucleic acids. As a part of a program of elucidating energetically favourable structures for double-stranded polynucleotides, an investigation of base-base interactions was undertaken. This was done in two parts: interactions (i) in free bases\* and (ii) in nearest neighbouring bases in regular polynucleotides (both single- and double-stranded). In this paper, we present the results of our calculations on stacking interactions in free bases. These have been compared with the stacking arrangements as found in single crystals.

Calculations of base-base interactions have been reported earlier<sup>1,2</sup> for Watson-Crick type of base-pairing in the double-

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\* Free base: two interacting bases not covalently linked by a sugar-phosphate backbone.

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helical geometry as given by Langridge *et al.*<sup>3</sup> To the best of our knowledge, the interactions for various relative positions and Orientations in all possible combinations of the bases have not been reported, except for the stacking of adenine bases in crystals<sup>4</sup>. So as a prelude to the study of base-base interactions in polynucleotides, we felt that it would be worthwhile to analyse the stacking patterns in free bases. In the subsequent paper, we will deal with the base-base interactions in polynucleotides (both single- and double-stranded).

#### METHOD OF CALCULATIONS

The base stacking between two bases has been referred to as the interaction between any two bases which are parallel or roughly so. Apart from the nature of the bases involved, the stacking interactions essentially depend on the interplanar distance between the bases at vertical planes and the relative positions and orientations of two interacting bases. These interactions can be calculated either in the monopole-monopole approximation<sup>2</sup> or in the dipole-dipole approximation<sup>1</sup>. Following the monopole-monopole approximation, the following four contributions to stacking interactions have been considered.

Monopole-monopole interaction: The bases with polar substituents have appreciable fractional charges which are asymmetrically distributed. Using the point charge approximation, the monopole-monopole interaction is given by

$$E_{MM} = \sum_{\substack{i \in A, \\ j \in B}} \frac{q_i q_j}{r_{ij}} \quad \dots\dots (1)$$

where  $q_i$  is the charge on the  $i^{\text{th}}$  atom of the base A,  $q_j$  is the charge on the  $j^{\text{th}}$  atom of the base B,  $r_{ij}$  the distance between the  $i^{\text{th}}$  atom of A and  $j^{\text{th}}$  atom of B.

Monopole-induced dipole interaction: The nucleic acid bases have polarizable rings and as such the point charges of the one base can polarize the other base and vice versa. The point charges in one base together can induce a dipole moment in the other base

and a dipole, so generated, can interact with the point charges to which it is due. The interaction depends on the molecular polarizability of the base involved and the point charges at the atomic centres. The expression is given by

$$E_{MD} = -\frac{1}{2} ( \alpha_A |\vec{\eta}_1|^2 + \alpha_B |\vec{\eta}_2|^2 ) \dots\dots (2)$$

where  $\alpha_A$  and  $\alpha_B$  are the molecular polarizabilities of A and B respectively.

$$\vec{\eta}_1 = \sum_{j \in B} \frac{q_j}{R_{A,j}^3} R_{A,j}$$

is the dipolar electric field at a point in A due to the J charges in B at corresponding distance  $R_{A,j}$ . Similarly, for

$$\vec{\eta}_2 = \sum_{i \in A} \frac{q_i}{R_{B,i}^3} R_{B,i}$$

$\alpha_A$  and  $\alpha_B$  are taken to be average isotropic molecular polarizability. This is a valid approximation since the calculations carried out by Pullman and Pullman<sup>2</sup> using both isotropic and anisotropic polarizabilities have indicated nearly the same results.

London-dispersion Interaction: Treating the base-base interaction as a molecular association, the above two types of interactions depend on the point charge distribution at the atomic centers of the bases. Since the charge distribution is not symmetrical, the interactions do not cancel out to zero. There is also a symmetrical type of interaction which does not depend explicitly on the charge distributions at atomic centers of the base, and the London-dispersion force is one such type of symmetric interaction.

The charge distribution in a molecule is the same as electron distribution and the electrons are not stationary but oscillate about a mean position such that the time and space average of the charge distribution gives net zero charge for the molecule at the mean position for an external observer. When two bases approach each other there are forced oscillations. That is,

one charge distribution oscillates in the field of the other and vice versa, giving rise to the well known London-dispersion forces due to the formation of a weak coupling between the two. Assuming that the charge distribution of the bases are due to an effective dipole, expression for the London-dispersion interaction is given as

$$E_{LD} = -\frac{3}{2} \alpha_A \alpha_B \left[ \frac{V_A V_B}{V_A + V_B} \right] \frac{1}{R_{AB}^6} \dots\dots (3)$$

where  $R_{AB}$  is the distance between the centres of the dipoles, in our calculation.  $V_A$  and  $V_B$  are the ionization potentials of the bases A and B respectively.

Repulsive Interaction: Two charge distributions cannot occupy the same place the same time. Therefore, there is a repulsive interaction between the two molecules which limits the closest possible distance  $R_{AB}$  between the two. The exact quantitative nature of molecular repulsion being not available, we have taken a trial function,

$$E_{RP} = \frac{D}{R_{AB}^{12}} \dots\dots (4)$$

and this interaction is also a symmetrical one as the London-dispersion interaction. The constant D is evaluated as follows.

Taking the symmetrical interaction as  $E_{sym} = (E_{RP} + E_{LD})$ , it is evident that this interaction is dependent on the geometrical overlap of the two bases at a given distance. This interaction will be maximum for maximal geometric overlap. Thus,  $E_{sym}$  will be maximum attractive when the bases are placed on top of each other such that the vertical distance  $R_0$  is close to twice the average van der Waal radii of the atoms of the base. If the average van der Waal diameter is  $3.35 \text{ \AA}$  then D is given by

$$\left( \frac{\delta E_{sym}}{\delta R_{AB}} \right)_{R_0} = 0 = C.R_0^6 - 2D$$

then,

$$D = \frac{C R_0^6}{2}, \text{ with } C = -\frac{3}{2} \alpha_A \alpha_B \frac{V_A V_B}{V_A + V_B} \dots\dots (4a)$$

It is to be noted that neither Devoe and Tinoco<sup>1</sup>, nor Pullman and Pullman<sup>2</sup> have considered the repulsive interaction. Obviously, they did not consider this necessary for calculations with fixed positions of the bases in single- or double-helical polynucleotide chains. However, Cleverie<sup>4</sup> et al. have considered the repulsion between two bases via atom-atom interaction. For the sake of simplicity, we have, as stated above, considered the repulsion term on the basis of molecular repulsion. Thus the total interaction, which is the sum of the four individual contributions mentioned above, is given by,  $E = E_{MM} + E_{MD} + E_{LD} + E_{RP}$ . The total interaction was considered in vacuum.

#### PARAMETERS VARIED IN THE CALCULATIONS

Relative positions and orientations of the two bases were varied with respect to four independent parameters as described in Fig.1. The parameter 'd' is varied from 4<sup>o</sup>A to 2.8<sup>o</sup>A. For a given (R,  $\theta$ ,  $\phi$ ) the total energy 'E' is always minimum around  $d = 3.3^{\circ}\text{A} - 3.4^{\circ}\text{A}$ , due to the particular choice of the constant 'D' in the expression (4). This, in fact, is consistent with the interplanar distance between two interacting bases in vertical planes, as observed in single crystals. Thus in this paper, we report interaction energies corresponding to  $d \approx 3.4^{\circ}\text{A}$ . The parameter 'R' was varied within a range such that there was always some geometric overlap between two interacting bases. For this purpose, two representative values of R, viz.  $R = 3.54^{\circ}\text{A}$  and  $R = 3.7^{\circ}\text{A}$ , were chosen. Both  $\theta$  and  $\phi$  were varied, at intervals of 30<sup>o</sup>, within a range between 0<sup>o</sup> to 360<sup>o</sup>. In tables I(a) and I(b), total interaction energies are given for various combinations of interacting bases, for  $\theta$  and  $\phi$  at intervals of 90<sup>o</sup>. In the range of 90<sup>o</sup>, the total energy of interaction is monotonic and does not undergo sharp fluctuations.

Most of the crystals of purines, pyrimidines, and related nucleosides and nucleotides are found either in mono-clinic or orthorhombic systems. So, for a single molecule in the

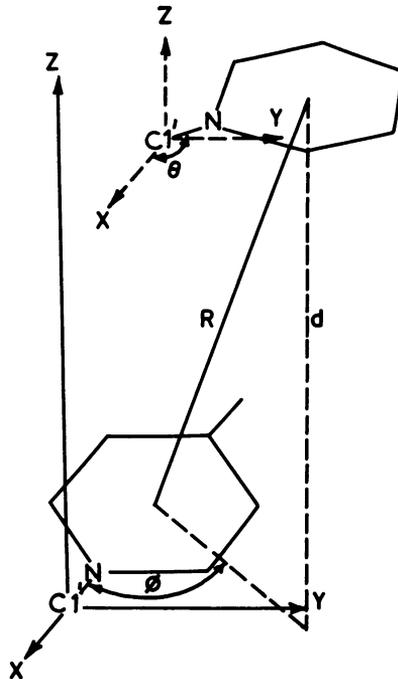


Fig.1 : Four parameters varied in the calculation

- d = Vertical distance between two bases stacked at parallel planes.
- R = Centre to centre distance between the two bases.
- $\theta$  = Angle in projection between two C1'-N bonds of the two bases, as shown.
- $\phi$  = Angle in project, between R and C1'-N bond of the lower base, as shown.

asymmetric unit, crystal symmetry demands that  $\theta$  can be either  $0^\circ$  or  $180^\circ$ . So in such cases,  $\phi$ -rotation plays a very important role for a given set of  $d$  and  $R$ . It picks up energetically most favourable stacking pattern corresponding to  $\theta = 0^\circ$  or  $180^\circ$ . On the other hand, when we have phospho-diester backbone covalently linking two neighbouring bases separated by interplanar distance of  $3.2^\circ\text{A} - 3.4^\circ\text{A}$ ,  $\theta = 0^\circ$  or  $180^\circ$  orientation is stereochemically impossible. In fact, we have found that the regions around  $\theta = 60^\circ$  and  $300^\circ$  for  $\phi = 90^\circ$  and  $270^\circ$  are stereochemically feasible. Thus, stacking interactions corresponding to these regions are relevant for regular polynucleotides.

BASE STACKING PATTERNS; NORMAL AND INVERTED TYPES:

For a given set of  $d, R, \theta$  and  $\phi$ , there can be two arrangements of stacked bases in vertical planes. These two types of arrangements are shown in Figs. 2a and 2b. For purines atoms  $C1'$ ,  $N9$  and

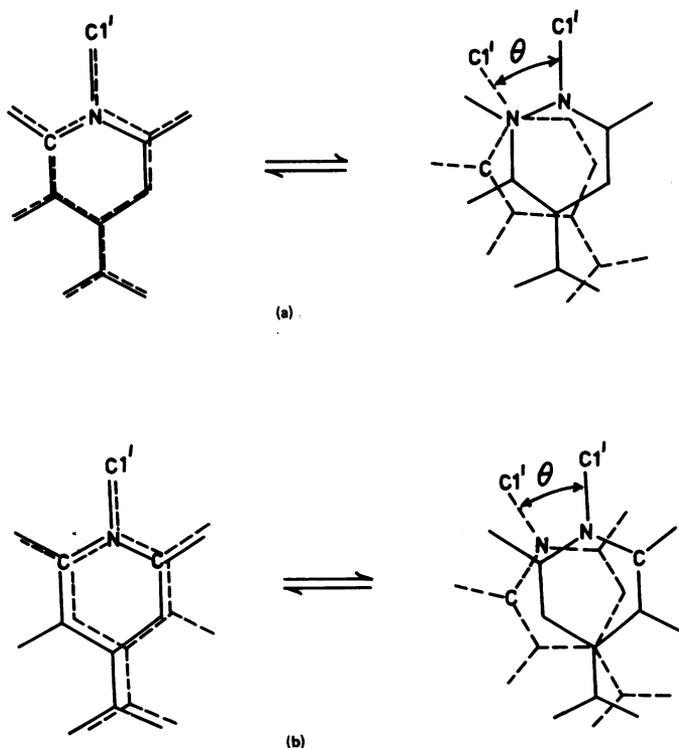


Fig. 2 : Schematic representation of normal and inverted stacking.

- a) Normal stacking: by making  $\theta$  equal to  $0^\circ$  and adjusting  $R$  and  $\phi$ , the two  $C1'-N$  bonds can be brought into coincidence and the two reference atoms  $C$  either coincide (for two identical bases) or lies on the same side of  $C1'-N$  bond (for non-identical bases).
  - b) Inverted stacking: by making  $\theta$  equal to  $0^\circ$  and adjusting  $R$  and  $\phi$  the two  $C1'-N$  bonds can be brought in coincidence but two reference atoms  $C$  lie on opposite sides of  $C1'-N$  bond.
- \* reference atom  $C$  will be  $C6$  in the case of pyrimidines and  $C8$  in the case of purines, similarly  $N$  will be  $N9$  for purines and  $N1$  for pyrimidines.

C8 were chosen as reference atoms and for pyrimidines they were atoms C1', N1' and C6. In normal stacking (Fig.2a), for two interacting pyrimidines (purines), the C1'-N1 (C1'-N9) bonds, can be made to coincide such that the C6 (C8) atoms of the two bases lie on the same side of C1'-N1 (C1'-N9) bonds, by making  $\theta = 0^\circ$  and followed by appropriate translation or vice versa. In the case of an inverted stacking arrangement (Fig.2b), one of the bases is rotated by  $180^\circ$  about C1'-N1 or C1'-N9 bond as the case may be from the normal type. Therefore, for such a stacking pattern, although the two C1'-N1 bonds for pyrimidines or C1'-N9 bonds for purines can be brought to coincidence with the reference atoms C6 or C8 lie on the opposite side of C1'-N1 or C1'-N9 bond.

### RESULTS:

The results of the calculations performed on G-G, A-A, T-T and C-C interactions for two types of stacking arrangements are presented in this section. And subsequently, the stacking arrangements, for two types corresponding to minimum energy are compared with the stacking patterns observed in single crystals of nucleic acid components. The calculations of stacking interactions between non-identical bases viz. G-C, A-T, A-G and C-T are in progress and will be reported later.

Calculations were done with methylated bases at the N-9 position for purines (A & G) and at the N1 position for pyrimidines (T & C). This was done so that free bases and bases in polynucleotides will have the same charge distribution. Hence, unless otherwise mentioned, whenever we state A, G, T & C we mean methylated bases. For a few specific cases the calculation was also done for nonmethylated bases.

Systematic features of stacking interactions for different bases are presented below.

#### G-G Stacking:

For normal stacking table I(a)) energy minimum was obtained for  $\theta = 180^\circ$  and  $\phi = 0^\circ$ , this arrangement is shown in Fig.3(a). Energy minimum due to inverted stacking (see table I(b)) corresponds to  $\theta = 0^\circ$  and  $\phi = 180^\circ$  (Fig.3(b)). In the crystal struc-

Table Ia: Normal Type

		$\theta$ / $\beta$	0°	90°	180°	270°
		0°	90°	180°	270°	
G-G Stacking	0°		+2.0(+2.6)	-11.7(-12.0)	-14.0(-13.3)	-9.0(-11.0)
	90°		+1.0(+1.8)	-13.0(-14.0)	-7.1(-9.6)	-11.1(-13.0)
	180°		+0.0(+0.9)	-11.5(-13.2)	-9.0(-11.6)	-13.8(-14.7)
	270°		+1.4(+2.0)	-9.9(-11.9)	-12.4(-12.7)	-10.4(-12.3)
A-A Stacking	0°		-7.9(-7.8)	-11.0(-12.1)	-8.6(-10.4)	-9.3(-10.9)
	90°		-6.6(-7.2)	-8.8(-10.3)	-8.4(-9.7)	-9.6(-10.8)
	180°		-6.6(-7.2)	-8.3(-9.9)	-8.9(-10.4)	-8.8(-10.4)
	270°		-9.0(-9.9)	-10.3(-11.4)	-12.5(-13.2)	-10.7(-11.5)
T-T Stacking	0°		-1.5(-1.4)	-6.9(-7.4)	-7.7(-7.7)	-4.9(-5.8)
	90°		-1.0(-1.2)	-5.0(-6.0)	-3.0(-4.6)	-7.5(-7.8)
	180°		-1.4(-1.4)	-1.9(-3.7)	-3.7(-4.8)	-4.4(-5.6)
	270°		-0.2(-0.6)	-5.6(-4.6)	-5.1(-6.3)	-3.6(-1.7)
C-C Stacking	0°		+4.8(+5.2)	-7.7(-8.8)	-10.5(-12.0)	-8.0(-8.8)
	90°		+3.5(+4.8)	-5.8(-7.2)	-15.9(-16.4)	-7.2(-8.3)
	180°		+5.3(+4.6)	-5.4(-6.9)	-14.0(-15.8)	-5.9(-7.3)
	270°		+2.7(+4.1)	-8.2(-9.0)	-7.2(-9.8)	-6.2(-7.5)

Table Ib: Inverted Type

		$\theta$ / $\beta$	0°	90°	180°	270°
		0°	90°	180°	270°	
G-G Stacking	0°		-10.0(-11.1)	-2.7(-2.2)	-3.3(-5.1)	-13.4(-14.0)
	90°		-8.1(-10.1)	-2.7(-6.7)	-5.9(-6.2)	-14.6(-15.0)
	180°		-12.0(-12.8)	-3.5(-3.8)	-5.3(-6.5)	-11.8(-13.1)
	270°		-12.0(-12.7)	-3.6(-4.0)	-5.6(-6.0)	-10.6(-12.1)
A-A Stacking	0°		-9.9(-11.1)	-10.0(-11.7)	-7.9(-9.3)	-9.3(-8.8)
	90°		-8.5(-9.8)	-8.9(-10.3)	-8.3(-9.5)	-7.9(-8.7)
	180°		-8.7(-10.0)	-7.9(-10.3)	-8.3(-9.6)	-6.6(-7.7)
	270°		-11.3(-12.1)	-11.0(-11.2)	-10.8(-11.2)	-9.0(-9.3)
T-T Stacking	0°		-4.1(-4.6)	-3.2(-3.8)	-8.0(-8.1)	-5.9(-6.8)
	90°		-4.6(-5.1)	-1.1(-2.1)	-4.4(-5.9)	-6.2(-6.6)
	180°		-4.0(-4.5)	-0.5(-1.6)	-2.5(-4.4)	-2.8(-6.4)
	270°		-1.5(-3.0)	-3.0(-3.6)	-3.7(-5.3)	-2.2(-6.3)
C-C Stacking	0°		-12.8(-13.4)	-6.2(-7.0)	-2.7(+2.7)	-6.7(-6.1)
	90°		-16.5(-16.0)	-6.5(-7.0)	+0.9(+2.6)	-4.4(-6.2)
	180°		-12.1(-13.0)	-6.6(-7.0)	+5.1(+4.1)	-6.6(-7.7)
	270°		-6.4(-8.9)	-6.4(-7.1)	+1.1(+1.9)	-7.4(-8.4)

Stacking energies in K calories per 2 moles of bases corresponding to  $d = 3.4^{\circ}\text{A}$  and  $R = 3.7^{\circ}\text{A}$ . Energy values given within the brackets correspond to  $d = 3.4^{\circ}\text{A}$  and  $R = 3.54^{\circ}\text{A}$ .

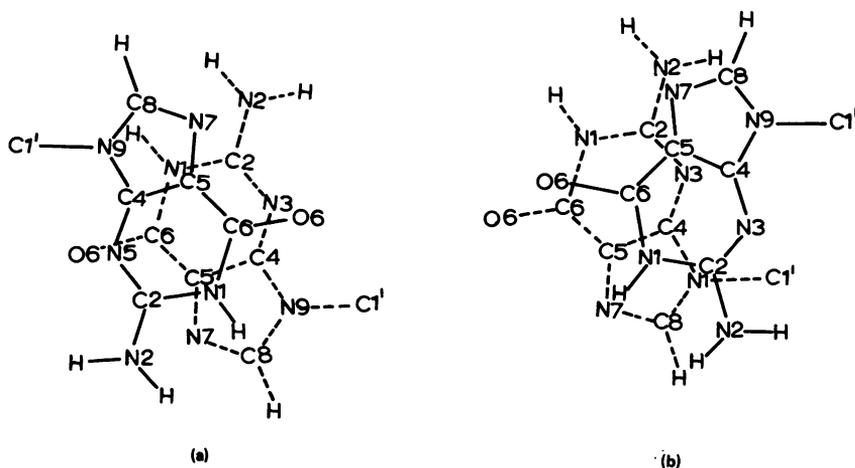


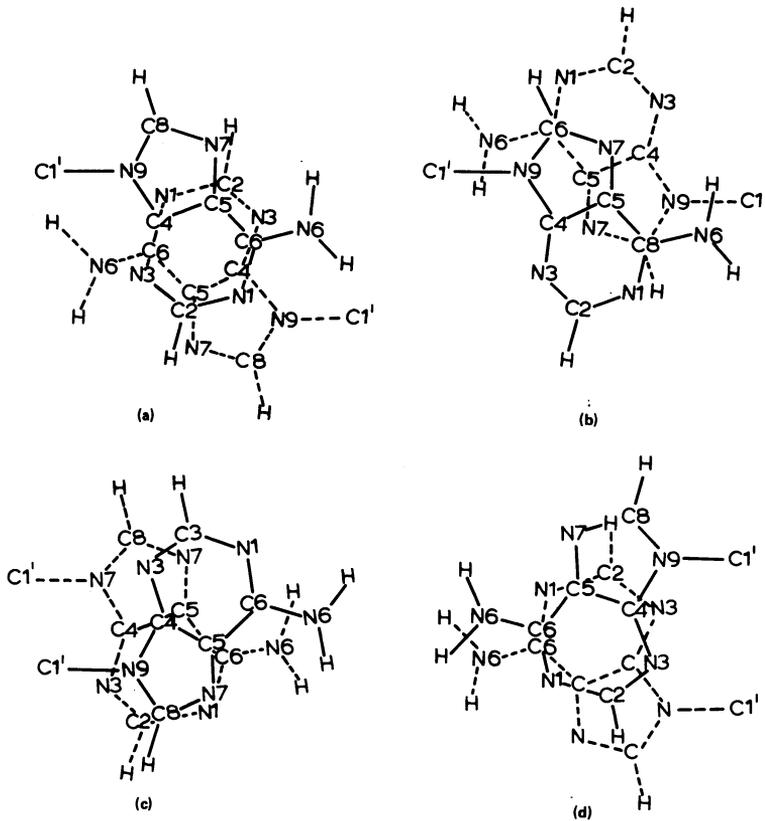
Fig.3 : Examples of G-G stacking patterns and the corresponding interaction energies.

- a) Normal stacking pattern corresponding to minimum energy; stacking energy,  $E = -14.0 \text{ Kcal/2 moles}$ ; stacking parameters,  $d = 3.4 \text{ \AA}$ ,  $R = 3.7 \text{ \AA}$ ,  $\theta = 180^\circ$  and  $\phi = 0^\circ$ .
- b) Inverted stacking pattern corresponding to minimum energy; observed in guanosine<sup>5</sup>; stacking energy,  $E = -12.0 \text{ Kcal/2 moles}$ ; stacking parameters,  $d = 3.4 \text{ \AA}$ ,  $R = 3.7 \text{ \AA}$ ,  $\theta = 0^\circ$  and  $\phi = 270^\circ$ .

ture of guanosine<sup>5</sup>, such a pattern of inverted stacking is observed which forms a H-bonded network. In the case of normal stacking, the arrangement ( $\theta = 180^\circ$  and  $\phi = 0^\circ$ ) corresponding to minimum energy cannot offer such a H-bonding scheme in keeping with the crystal symmetry. However, such a H-bonded network is possible for normal stacking when  $\theta = 0^\circ$  and  $\phi = 0^\circ$  with non-methylated guanine<sup>6</sup>. In the co-crystal of 9-Et G+5-Met C<sup>7</sup> (1:1 complex), normal stacking arrangement for  $\theta = 180^\circ$  and  $\phi = 90^\circ$  orientation is found. Such an arrangement is further stabilized by Watson-Crick base-pairing between G and C.

#### A-A Stacking:

From table I(a), it is seen that energetically most favoured normal stacking arrangement is obtained for  $\theta = 180^\circ$  and  $\phi = 270^\circ$ . The stacking pattern is given in Fig.4(a); it is identical with the stacking arrangement in the crystal structure of 9-Met-A<sup>8</sup>.



**Fig.4 :** Examples of A-A stacking patterns and the corresponding interaction energies.

- a) Normal stacking pattern corresponding to minimum energy; observed in 9-MetA<sup>8</sup>; stacking energy,  $E = -12.5$  Kcal/2 moles; stacking parameters  $d = 3.4^{\circ}\text{A}$ ,  $R = 3.7^{\circ}\text{A}$ ,  $\theta = 180^{\circ}$  and  $\phi = 270^{\circ}$ .
- b) Normal stacking pattern; observed in 9-EtA+1-Met-5FU<sup>9</sup> (1:1 complex); stacking energy,  $E = -8.9$  Kcal/2 moles; stacking parameters,  $d = 3.4^{\circ}\text{A}$ ,  $R = 3.7^{\circ}\text{A}$ ,  $\theta = 180^{\circ}$  and  $\phi = 90^{\circ}$ .
- c) Inverted stacking pattern; observed in ApU<sup>10</sup> in two As; stacking energy,  $E = -11.1$  Kcal/2 moles; stacking parameters,  $d = 3.4^{\circ}\text{A}$ ,  $R = 3.54^{\circ}\text{A}$ ,  $\theta = 0^{\circ}$  and  $\phi = 0^{\circ}$ .
- d) Inverted stacking corresponding to minimum energy; stacking energy,  $E = -11.3$  Kcal/2 moles; stacking parameters,  $d = 3.4^{\circ}\text{A}$ ,  $R = 3.7^{\circ}\text{A}$ ,  $\theta = 0^{\circ}$  and  $\phi = 270^{\circ}$ .

Although the normal stacking arrangement for  $\theta = 180^\circ$  and  $\phi = 90^\circ$  orientation (see Fig.4(b)) has lower stabilization energy (see table I(a)), the crystal structure of 9-Et-A + 1 Met-5-FU<sup>9</sup>, has this orientation of the adenine molecules due to Hoogsteen type of base-pairing between A and U. Inverted stacking is observed in the crystal structure of ApU<sup>10</sup> between two neighbouring adenine molecules, in the structure; such a pattern is shown in Fig.4(c). Energy of stacking interaction corresponding to such an arrangement is within 1 kcal from the minimum energy (table I(b)). Inverted stacking pattern corresponding to minimum energy is given in Fig.4(d). Both normal and inverted stacking pattern corresponding minimum energy, can offer the same H-bonding network as observed in 9-EtA. However, close packing is only observed for normal stacking.

### T-T Stacking:

Energy minimum for the normal stacking corresponds to ( $\theta = 180^\circ$ ,  $\phi = 0^\circ$ ) orientation, as seen from table I(a) the stacking pattern is identical with those observed for the crystal structure of 1-Met+9-EtA<sup>11</sup> (1:1 complex) as mentioned in Fig. 5(a). Hoogsteen base-pairing between A and T further stabi-

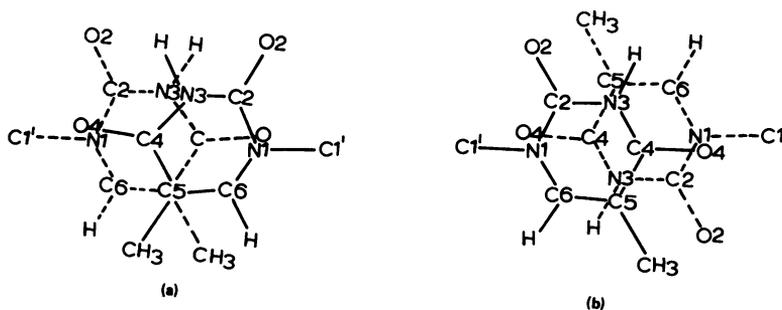


Fig. 5: Examples of T-T stacking patterns and the corresponding interaction energies.

- a) Normal stacking pattern corresponding to minimum energy, observed in 9-EtA+1-MetT<sup>11</sup> (1:1 complex); stacking energy,  $E = -7.7$  Kcal/2 moles; stacking parameters,  $d = 3.4^\circ\text{A}$ ,  $R = 3.7^\circ\text{A}$ ,  $\theta = 180^\circ$  and  $\phi = 0^\circ$ .
- b) Inverted stacking pattern corresponding to minimum energy; stacking energy,  $E = -8.0$  Kcal/2 moles; stacking parameters,  $d = 3.4^\circ\text{A}$ ,  $R = 3.7^\circ\text{A}$ ,  $\theta = 180^\circ$  and  $\phi = 0^\circ$ .

lizes the stacking pattern. Stacking arrangement of T-monohydrate<sup>12</sup> is of normal type, but it takes up ( $\theta = 0^\circ$ ,  $\phi = 90^\circ$ ) orientation where bases are very poorly stacked. The stacked bases gain energy due to a series of H-bonds. Such a H-bonding scheme is not possible for methylated thymine. It is seen from table I(b), that the minimum energy for inverted stacking arrangement is obtained for ( $\theta = 180^\circ$ ,  $\phi = 0^\circ$ ) orientation (Fig.5(b)) where H-bonding is possible between the bases.

### C-C Stacking:

Table I(a) shows that for normal stacking, minimum energy corresponds to ( $\theta = 180^\circ$ ,  $\phi = 90^\circ$ ) orientation as shown in Fig. 6(a). Such an arrangement is again stabilized by Watson-Crick base-pairing between G and C. Although, for normal stacking for  $\theta = 0^\circ$  for all  $\phi$ , is energetically unfavourable, such a stacking pattern is observed for non-methylated cytosine due to formation of a H-bonded network. For inverted stacking ( $\theta = 0^\circ$ ,  $\phi = 90^\circ$ )

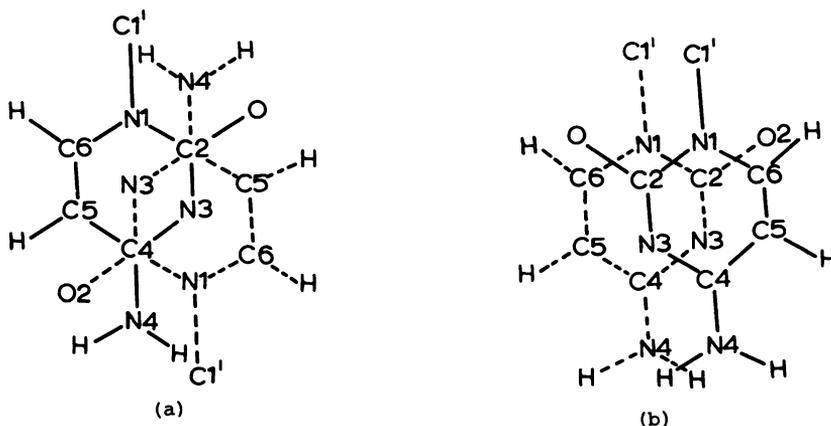


Fig. 6: Examples of C-C stacking patterns and the corresponding interaction energies.

- a) Normal stacking pattern corresponding to minimum energy; observed in 9-EtG+1-MetC<sup>7</sup> (1:1 complex); stacking energy,  $E = -15.9$  Kcal/2 moles; stacking parameters,  $d = 3.4^\circ\text{A}$ ,  $R = 3.7^\circ\text{A}$ ,  $\theta = 0^\circ$  and  $\phi = 90^\circ$ .
- b) Inverted stacking pattern corresponding to minimum energy; observed in C-monohydrate<sup>13</sup>; stacking energy,  $E = -16.5$  Kcal/2 moles; stacking parameters,  $d = 3.4^\circ\text{A}$ ,  $R = 3.7^\circ\text{A}$ ,  $\theta = 0^\circ$  and  $\phi = 90^\circ$ .

orientation correspond to minimum energy of interaction, such a stacking pattern is shown in Fig.6(b).

Conclusions:

The main conclusions from the present study are as follows:

(i) We have defined two types of stacking patterns, normal and inverted, which are energetically almost equally favourable. Both the types are seen in A-A, G-G, T-T and C-C stacking arrangements in single crystals.

(ii) Stacking interaction depends upon two factors, namely, geometric overlap (decided by  $d$  &  $R$ ) of the stacked bases at vertical planes which contributes to  $E_{\text{sym}} = E_{\text{LD}} + E_{\text{RP}}$  and the relative positions and orientations of the atoms (dependent on  $\theta$  &  $\phi$  for a given  $d$  &  $R$ ) of the stacked bases which govern  $E_{\text{asym}} = E_{\text{MM}} + E_{\text{MD}}$ . For a given geometric overlap, we can have different relative positions and orientations of the atoms in the two bases. In some orientations,  $E_{\text{asym}}$  can be attractive and in some other it can be repulsive, whereas  $E_{\text{sym}}$  is always attractive for the values of  $D$ ,  $R$  chosen. Therefore, the stacking interaction becomes a combined effect of  $E_{\text{sym}}$  and  $E_{\text{asym}}$ . That is the reason why we see a variation of total stacking energy 'E' for various  $(\theta, \phi)$  at a given set of  $d, R$ . Most favourable stacking interaction at a given  $(d, R)$  is due to the most attractive  $E_{\text{asym}}$ . This occurs mostly because of the proximity of unlike charges in the two bases. Thus, highest geometric overlap between the bases need not necessarily mean the most favourable stacking interaction.

(iii) H-bonding plays an important role in deciding a stacking pattern. H-bonding can have compensating or additive effect. For example, it has a compensating effect in stabilizing a stacking pattern otherwise unfavourable (viz. stacking of nonmethylated T-T, C-C and G-G). In such cases, stacking patterns corresponding to minimum energy fail to offer such a H-bonding scheme, so that the total energy of stabilization in the former is always higher. In some cases (viz. G-G stacking in guanosine etc.), H-bonding serves an additive effect. Here, stacking pattern corresponding to minimum energy can also form a neat H-bonding

network consistent with the crystal symmetry. But in the cases where we have no compensating feature like H-bonding, the stacking patterns invariably correspond to minimum energy (or close to it) (viz. G-G inverted stacking in GpC<sup>14</sup>, A-A inverted stacking in ApU etc.).

There are two major restrictions on the stacking pattern of free bases in crystals, namely, crystal symmetry and H-bonding. On the other hand, the stereochemistry of the backbone and H-bonding impose restrictions on the stacking patterns of bases in the next paper.

#### References:

1. De Voe, H., and Tinoco, I. Jr. (1962) *J. Mol. Biol.* 4, 500-511
2. Pullman, A. and Pullman, B. (1968) *Adv. Quant. Che.*, 4, 267-321.
3. Langridge, R., Marvin, A., Seeds, A.W., Wilson, H.R., Hooper, C.W., Wilkins, M.H.F., and Hamilton, L.D. (1960) *J. Mol. Biol.*, 2, 38-64.
4. Gaillet, J., and Claverie, P. (1974) *Biopolymer*, 13, 604-614.
5. Steward, R.F., and Jensen, L.H. (1964) *J. Chem. Phys.*, 40, 2071-2075.
6. Tomita, K., Katz, L. and Rich, A. (1967) *J. Mol. Biol.* 30, 545-549.
7. O'Brien, E.J. (1967) *Acta Cryst.*, 23, 92-106.
8. Thewalt, U., Bugg, C.E., and Marsh, R.E. (1970) *Acta Cryst.*, B26, 1089-1101.
9. Bugg, C.E., Thewalt, U. and Marsh, R.E. (1968) *Biochem. Biophys. Res. Communication*, 33, 436-440.
10. Seeman, N.C., Rosenberg, J.M., Suddath, F.L., Kim, J.J.P. and Rich, A. (1976A) *J. Mol. Biol.*, 104, 109-144.
11. Hoogsteen, K. (1963), *Acta Cryst.*, 16, 907-916.
12. Gerdil, H. (1961), *Acta Cryst.*, 14, 333-344.
13. Jeffrey, G.A. and Kinoshita, Y. (1963) *Acta Cryst.*, 16, 20-28.
14. Rosenberg, J.M., Seeman, N.C., Roberta, O.D. and Rich, A. (1976), *J. Mol. Biol.*, 104, 145-167.